Mini, Midi or Maxi GeBaFlex-tube

Protein, DNA and RNA Extraction Protocols

Protein Extraction from Polyacrylamide Gel

The following protocols describe the use of GeBaFlex-tubes for electroelution of proteins or protein complexes from a variety of gels. Electroelution can be performed with non-denaturing and denaturing (SDS) polyacrylamide gels, one-dimensional and two-dimensional polyacrylamide gels, and isoelectric focusing gels.

Avoid fixation of proteins before electro elution (e.g. fixation with methanol, acetic acid, etc). **Fixation will greatly reduce extraction yield**. SeeBand Reagent (Gene Bio-Application Ltd, Cat. No. SB010) is a sensitive Coomassiebased reagent for staining polyacrylamide gels and is compatible with extraction. SeeBand does not require mixing or preparation, fixation, or destaining, and stains protein within 5-10 min.

GeBaFlex-tubes can be used to extract proteins for analysis by MALDI-MS (Matrix-assisted Laser Desorption/Ionization Mass Spectrometry). The GeBaFlex-tubes enable high protein recovery, and the MS precipitation buffer (See Ordering Information) thoroughly removes SDS. This can facilitate more sensitive MALDI-MS analysis. If analysis by MALDI-MS will be performed, follow the electroelution protocol below with the Notes for MALDI-MS.

Procedure

1. Fill GeBaFlex-tube with deionized water; see Table 1 for recommended volumes. Incubate for at least 5 min. Check that there is no water leaking from the tube. The water level will decrease as dry membranes absorb some of the water.

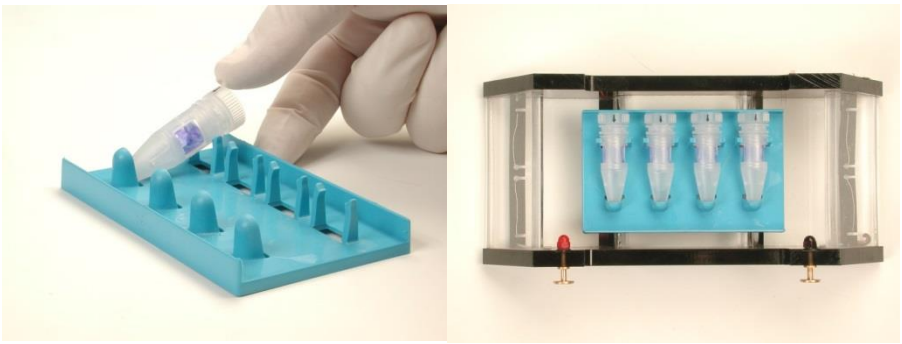
   Table 1

GeBaFlex-tube	Volume of deionized water	Maximum size gel slice	Running buffer added to tube
Mini	0.25 ml	0.4 cm x 1.1 cm	0.2 ml
Midi	0.8 ml	0.5 cm x 1 cm	0.7-0.8 ml
Maxi	3.0 ml	2 cm x 1 cm	2.5-3.0 ml

2. After staining gel, excise the gel slice containing the protein fragment with a clean, sharp scalpel. Minimize the size of the gel slice by removing extra gel.
3. Remove water from the tube. Transfer gel slice to a GeBaFlex-tube. The maximum gel slice capacity per device is indicated in Table 1. Don't fill tube with several gel slices. Fill tube with protein-running buffer (see Table 1 for recommended volumes). Typical SDS-PAGE running buffers may be used for standard MS applications; see below for running buffer composition when samples are to be analyzed using MALDI-MS. Avoid air bubbles in the tube. Close the tube gently.

   Not for MALDI-MS: A standard protein running buffer for MALDI-MS is 250 mM Tricine, 25 mM Tris-base, 0.025% SDS, pH 8.5
4. Place tube in the appropriate size Supporting Tray (See Figure 1A below). **The two membranes of each GeBaFlex-tube must be perpendicular to the electric field to permit electric current to pass through the tube**.

Note: The supporting trays can hold 1–4 Mini or Midi, or 1–3 Maxi GeBaFlex-tubes.



A **B**

Figure 1: (A) Insertion of Midi GeBaFlex-tube in Midi Supporting Tray showing correct orientation of membranes, which must be perpendicular to the electric field. (B) Supporting Tray containing four GeBaFlex-tubes in a horizontal electrophoresis tank. Membranes of each tube are perpendicular to the electric field.

5. Place Supporting Tray containing GeBaFlex-tube(s) in a horizontal electrophoresis tank containing protein running buffer (see Figure 1B). Fully immerse GeBaFlex-tube(s) in the buffer.

6. Apply electric current (usually 100 V) until the protein exits the gel slice. The optimum electroelution time will need to be determined for each sample and gel concentration. The minimum electroelution time for BSA (66 kDa) from a 10% SDS-PAGE gel is at least 85 min. For general electroelution times see Table 2.

Note for MALDI-MS: Due to the lower amount of SDS generally used for samples that will be analyzed by MALDI-MS, the usual electric current for elution is 150 V. The minimum electroelution time for BSA from a 10% SDS-PAGE under these conditions is at least 2 h.

Table 2: Electroelution times

Protein (kDa)	Time (min)		
	Mini	**Midi**	**Maxi**
14	30-40	35-45	50-60
18	35-45	45-50	55-65
25	40-50	50-55	70-80
29		55-65	
35	50-60		110-120
40		60-70	
45	55-65	65-75	130-140
50		75-85	
66	70-80	85-95	150-160
81		105-115	
116	90-100	120-130	180-190
128		140-150	

7. Release protein from membrane by reversing polarity of electric current for 2 min.
8. Gently open GeBaFlex-tube. Pipet protein-containing solution up and down at least 5 times on the inner side of the membrane. Transfer solution to clean microcentrifuge tube.
9. Centrifuge the microcentrifuge tube for 1 min at maximum speed to pellet gel residues.
10. Transfer supernatant to clean microcentrifuge tube. The protein can be used immediately, concentrated by standard concentration methods, precipitated (see Protein Precipitation protocol) or dialyzed. If desired, the same GeBaFlex-tube used for electroelution may be used for dialysis. Carefully remove gel slice, taking care not to puncture the membranes. See Dialysis or Sample Concentration protocol.

Protein precipitation

1. Add 1 volume 20% TCA (See ordering information below) to sample. Mix thoroughly by vortexing.

   Optional: For samples to be analyzed by either standard MS or MALDI-MS, begin by adding 0.l vol MS buffer. Mix thoroughly, incubates for 15 min at room temperature and then add 0.5 vol 20% TCA (for standard MS) or 0.2 vol 50% TCA (for MALDI-MS). Mix thoroughly by vortexing and proceed to Step 3.
2. Incubate 1 hr in 4^{o}C.
3. Centrifuge sample at 4^{o}C for 30 min at 14,000 RPM. Decant supernatant carefully.
4. Add cold 100% acetone to wash the pellet (e.g. use 0.5-2 ml acetone depending on pellet size). Mix thoroughly by vortexing.
7. Incubate at -20^{o}C for 30 min. Incubating samples at –20°C overnight can increase protein precipitation efficiency.
8. Centrifuge at 4^{o}C for 30 min at 14,000 RPM. Decant supernatant and air-dry the pellet.
9. Resuspend pellet in an appropriate volume 0.1M NaOH or deionized water. If deionized water is used, incubate sample for 5 min. at 60^{o}C, resuspend the sample and incubate 5 min. more at 60^{o}C.

   Note for MALDI-MS: Resuspend pellet in an appropriate amount of solution suitable for MALDI-MS. The characteristics of the protein are important for determining the appropriate solution.

DNA and RNA Extraction from Gel

Use the following protocol for extracting DNA or RNA from polyacrylamide or agarose gels.

Procedure

1. Add appropriate volume of deionized water (see Table 1 for recommended volumes). Incubate for at least 5 min. empty the tube. Carefully check that there is no water leaking from the tube. The water level will decrease as dry membranes absorb some of the water.
2. Excise gel slice containing desired DNA or RNA fragment using a clean, sharp scalpel. Trim away excess gel.
3. Transfer gel slice to GeBaFlex-tube. The maximum gel slice capacity per tube is indicated in Table 1. Fill the tube with running buffer; the same that was used in the gel separation

step, to top of the membranes (see Table 1 for recommended volumes). Avoid introducing air bubbles in the tube. Gently close tube.

4. Place tube in the Supporting Tray (See Figure 1A). The two membranes of GeBaFlex-tube must be positioned perpendicular to the electric field to permit electric current to pass through tube.
5. Place supporting tray containing GeBaFlex-tube(s) in a horizontal electrophoresis tank containing running buffer (see Figure 1B).
6. Apply electric current (usually at 80-150 volt) until nucleic acid exits gel slice. The optimum electroelution time must be determined for each sample and gel concentration. For general electroelution times see Tables 3-4.

Table 3: Minimum electroelution times required to elute various DNA fragments from 4% PAGE at 100-150 volts.

DNA Fragment size (bp)	Time (min)		
	Mini	Midi	Maxi
100	10-15	10-20	10-15
200	15-20		15-20
300		15-25	
500	30-35	20-30	30-35
800		25-35	
1000	55-60		55-60
1050		30-40	
1400	75-80		75-80
2700		45-55	

Table 4: Minimum electroelution times required to elute various RNA fragments from 4% PAGE at 100-150 volts.

RNA Fragment size (nt)	Time (min)
	Midi
100	15-25
400	25-35
600	35-45
1000	45-55

7. Release nucleic acid from membrane by reversing polarity of electric current for 2 min.
8. Gently open GeBaFlex-tube. Pipet eluate up and down at least 5 times on inner side of membrane, taking care to avoid gel slice and to not puncture the membrane. Transfer eluate to clean microcentrifuge tube.
9. Centrifuge eluate for 1 min at maximum speed to pellet gel residues.
10. Transfer supernatant to clean microcentrifuge tube. Concentrate nucleic acids using standard precipitation protocols.

For ordering information visit: http://www.geba.org/apage/117431.php